CINT Science Highlight – September 22, 2010

NanoCluster Beacon detects specific nucleic acid target sequence for diagnostics

The detection and quantification of specific biomolecules, ions, or metabolites are important for *in vivo* real-time monitoring of cellular processes and for *in vitro* biosensing and clinical diagnosis. Fluorescent probes that enable detection without separation are desirable for intracellular studies, where removal of unbound probes is difficult. Molecular beacons are hairpin-shaped nucleic acid probes that fluoresce upon hybridization with specific nucleic acid targets. While molecular beacons are some of the most successful separation-free probes, they have drawbacks for fluorescence enhancement.

Center for Integrated Nanotechnologies (MPA-CINT) scientists have demonstrated that DNA-templated silver nanoclusters (DNA/Ag NCs) can be used to detect specific nucleic acid targets. The nanoclusters circumvent many of the shortcomings of conventional molecular beacons. Hsin-Chih (Tim) Yeh, Jaswinder Sharma, Jason Han, Jennifer Martinez and James Werner have developed a novel way to control conversions of DNA-templated silver nanoclusters (DNA/Ag NCs) between highly fluorescent and weakly fluorescent states. This reversible fluorescence enhancement is created through close proximity of the nanoclusters with guanine-rich DNA. Based upon this enhancement mechanism, the researchers created a new molecular probe, termed a NanoCluster Beacon, which can detect specific nucleic acid target sequences. The team demonstrated sensitive and quantitative detection of an influenza DNA target and the detection of a human oncogene sequence. Because the probe "lights up" upon binding target DNA, there is no need to remove the unbound probes. This makes it useful for situations where removal of unbound probes is challenging. Because the fluorescence enhancement is caused by an intrinsic nucleobase (guanine), this new probe is simple, inexpensive, and compatible with commercial DNA synthesizers.

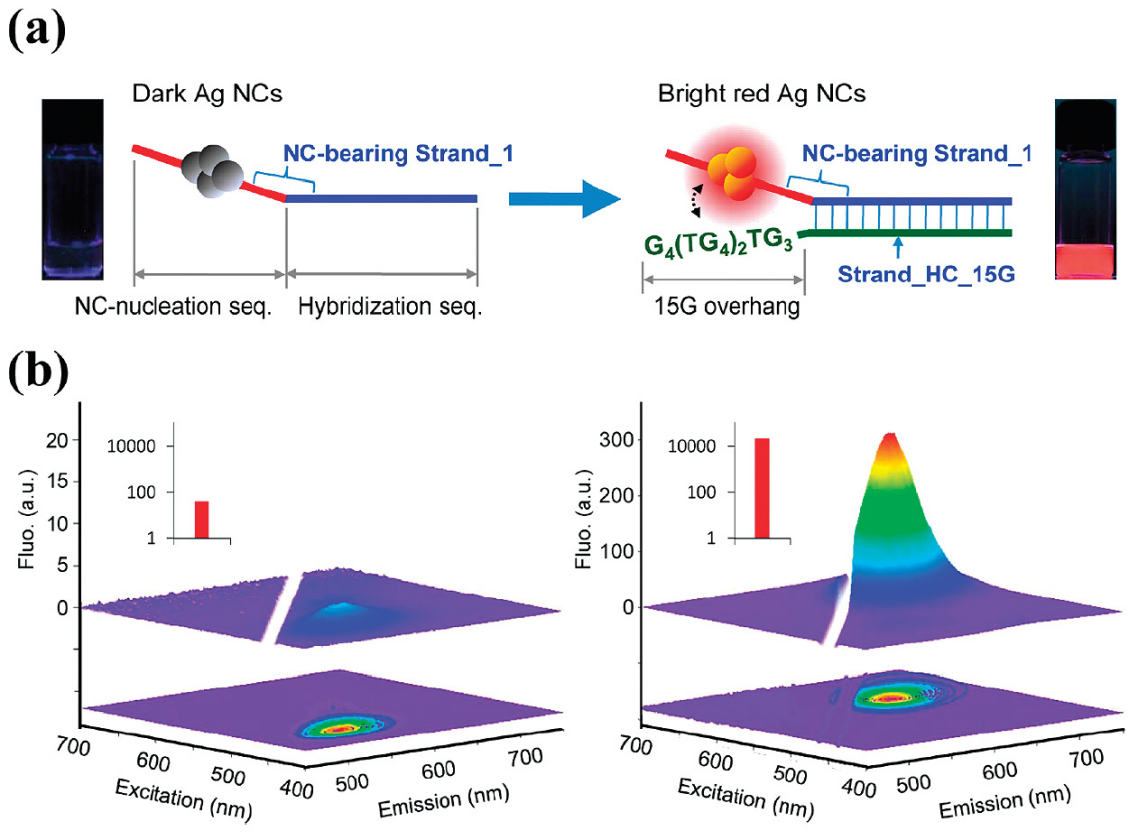

Figure 5. Schematic and data showing the red fluorescence enhancement of DNA templated silver nanoclusters (DNA/Ag NCs) caused by guanine proximity. (a) Schematic depicting red fluorescence enhancement of DNA/Ag NCs through proximity with a G-rich overhang, 3′-$G_4(TG_4)_2TG_3$, caused by DNA hybridization, and photographs of the resulting emission under ultraviolet (366 nm) irradiation. (b) 3D- and 2D-contour plots of excitation/emission spectra of the Ag NCs before (left) and after (right) hybridizing NC-bearing Strand_1 with Strand_HC_15G. Inset: Integrated red fluorescence emission with the buffer fluorescence subtracted in arbitrary units. The excitation/emission peaks for aged NCs on Strand_1 before hybridization were at 460 nm/543 nm. The excitation/emission peaks changed to 580 nm/636 nm after hybridization. The integrated red fluorescence emission was enhanced 500-fold after duplex formation.

This study is the first example of using spectral conversions of (DNA/Ag NCs) for the specific detection of DNA targets in a separation-free format. The journal ***Nano Letters*** published the research. Immediate applications of NanoCluster Beacons include using it as a readout mechanism for DNA microarrays and single-molecule-based digital assays. The Laboratory has filed a provisional patent application, and the scientists are working with Technology Transfer Division to commercialize this technology. The LANL Laboratory Research and Development (LDRD) program funded the work. The scientists conducted the research at the Center for Integrated Nanotechnologies, a DOE Office of Basic Energy Sciences user facility at LANL. Reference: "A DNA−Silver Nanocluster Probe That Fluoresces upon Hybridization," ***Nano Letters*** *10*, 3106 (2010); doi: 10.1021/nl101773c. Online: pubs.acs.org/doi/full/10.1021/nl101773c. Technical contact: *James Werner*